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REVIEW

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Sequence and function of the two P domain potassium channels: implications of an emerging superfamily

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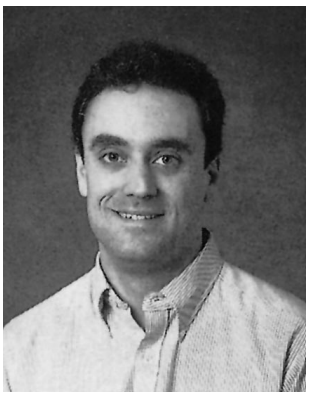

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Abstract A new superfamily of K^+ channels has emerged in the past 2 years. Notable for possessing two pore-forming P domains in each subunit, members of the superfamily have been recognized through phylogeny from micro-organisms to humans. Four subfamilies of two P domain channels have been isolated thus far; among these are the first cloned examples of outward rectifier and open rectifier (or leak) K^+ channels. The two P domain K^+ channels offer a new perspective from which to glimpse the molecular basis for function and dysfunction of K^+-selective ion channels.

Key words Potassium channel · Two P domains · *TOK1* · d-ORK1 · HOHO1 · h-TPKC1

Abbreviations *NCBI* National Center for Biotechnology Information

Introduction

Ion channels are proteins that reside in the plasma membranes of all cells and control their electrical activity [1]. In response to stimuli, such as neurotransmitters, mechanical stress, and voltage changes, channels open a water-filled pore across the membrane through which selected ions passively diffuse [2]. In this simple fashion ion channels mediate rapid signaling events that grant us sight, sensation, movement, and thought and execute slower, but essential, cellular house-keeping duties such as fluid and electrolyte homeostasis.

The first K^+ channel gene to be isolated, Shaker, was identified as the cause of a motion disorder in fruit flies [3–5]. Over 80 related K^+ channel genes have been cloned based on their sequence homology to Shaker, each encoding a protein with a single pore-forming P domain and six probable transmembrane segments (1P/6TM protein subunits) [6]. More recently, genes for K^+ channels have been isolated by expression cloning, a method that does not select for homology but identifies genes whose products show ion channel function [7–10],

or by computer searches for channel-like motifs in the expanding global database of nucleotide and protein sequences [11, 12]. It is by these later methods that members of the two P domain superfamily of K^+ channels were discovered.

Here we describe examples of each of the four recognized lineages of the new superfamily. *TOK1* was the founding member of the superfamily [11]. Isolated from *Saccharomyces cerevisiae*, it is an outwardly rectifying K^+ channel with two P domains and eight proposed transmembrane segments (2P/8TM). d-ORK1 was isolated from *Drosophila melanogaster* [10]; it is an open-rectifier (or leak) K^+ channel with two P domains and only four proposed transmembrane segments (2P/4TM). h-TPKC1, from human brain, shares a probable 2P/4TM structure with d-ORK1 but, like Tok1, is an outward rectifier [13]. Finally, HOHO1 was cloned from human brain and has a probable 2P/4TM structure (this report); we find it to be nonfunctional in oocytes while others report that it is a weak inward rectifier [12]. This growing superfamily of two P domain K^+ channels is examined below in the context of knowledge about K^+ channels with a single P domain.

Potassium channel function

Potassium channels are both efficient and highly selective; some catalyze flux of 100 million K^+ ions each second through a single channel complex while passing only one Na^+ ion in error every 10,000 events [14]. Because K^+ ions are the predominant monovalent cation in mammalian cells, and extracellular K^+ concentrations are low, opening K^+ channels favors an outpouring of these positively charged ions at the positive voltages characteristic of excitation. Outflow of K^+ ions shifts the voltage across the cell membrane toward the equilibrium reversal potential for K^+ ions (E_K), that is, the voltage where the tendency for K^+ ions to move outwardly (down their concentration gradient) is balanced by their tendency to move inward (down their electrical gradient). By this mechanism open K^+ channels mediate recovery after activity in excitable tissues such as nerves, skeletal muscles, and the heart and stabilize cellular potential near E_K [15]. Before their genes were cloned, individual K^+ channels were studied in native tissues and categorized by their functional attributes [16, 17]. Cloning of genes for single P domain K^+ channels has revealed examples of many but not all functional categories of K^+ channels; numerous voltage-gated, inward-rectifier, and ligand-gated K^+ channels are now known.

Voltage-gated K^+ channels

Voltage-activated K^+ channels undergo changes in protein conformation in response to changes in transmembrane potential to produce an open channel state that allows ion permeation (Fig. 1). Channels in this group show marked variation in opening and closing rates, and this underlies their wide diversity of roles in vivo. *Delayed rectifiers* activate with a delay after membrane depolarization and close only after cells return to resting potential. By opening relatively slowly, these channels do not inhibit the early explosive rising phase of the action potential but act in a delayed fashion to repolarize cells and prepare them for subsequent stimulation (Fig. 1b). On the other hand, *A currents* are voltage-gated channels that inactivate rapidly after opening, bringing channels to a nonconducting state unique from the closed state (Fig. 1a). These channels control the rate at which cells reach firing threshold and thus the interval between excitatory events. Both delayed rectifier and A-type voltage-gated K^+ channel subunits have a 1P/6TM topology (Fig. 1d). Indeed, these two phenotypes can be interconverted by site-directed mutations [18], heteromultimeric channel formation [19–21], and interaction with accessory subunits (see below) to alter the kinetics of inactivation.

Heteromeric subunit association is required for function of the slow delayed rectifier of human heart I_{Ks} [22–25] and perhaps for its rapid counterpart I_{Kr} as well [26]. These channels form through association of 1P/6TM protein subunits (KvLQT1 and HERG, respectively) and minK, a unique 130 residue protein with one transmembrane stretch that appears to contribute to for-

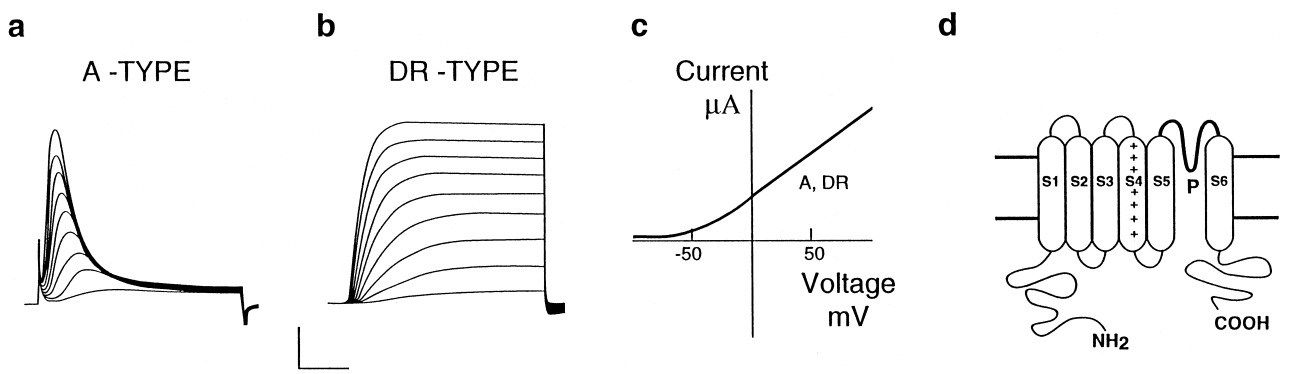

Fig. 1a–d Drawings to represent common attributes of voltage-gated K^+ channels. **a** Idealized A-type currents in whole oocytes in response to a family of voltage pulses from a holding voltage of –80 mV to command voltages of –40 to +40 mV in 10-mV steps. **b** Idealized delayed rectifier currents subjected to the protocol in **a**. **c** Peak current/voltage relationships. **d** Probable 1P/6TM membrane topology. *Scale bars*, 1 μA and 5 ms

mation of the channel pore [27]. Mutations in KvLQT1 or HERG that decrease channel function and slow cardiac repolarization have been found in some patients with genetically determined "long QT syndrome," a dysrhythmia that prolongs action potential duration, predisposing to torsade de pointe and sudden death [28–31].

Inward rectifier K^+ channels

Ion channels that preferentially pass K^+ ions inward are called inward rectifier K^+ channels. They show a steep voltage dependence, but unlike voltage-gated K^+ channels which have a fixed response to voltage, inward rectifier channels shift the voltage at which they pass current with changes in external K^+ ion concentration (Fig. 2c). This reflects the mechanism underlying opening and rectification in these channels – intracellular magnesium and polyamines tonically occlude the ion conduction pore and are expelled only when voltage or changing K^+ ion concentration favors inward movement of K^+ ions. Inward rectifiers stabilize the membrane near E_K and thus counter the hyperpolarizing effects of electrogenic sodium pump activity and the depolarizing effects of pacemaker currents [1]. These K^+ channel subunits have a 1P/2TM topology (Fig. 2d). A unique inward rectifier from renal cortical tubular cells that exhibits marked sensitivity to pH, RACTK1, has a modified P domain and two transmembrane stretches [32, 33].

Inwardly rectifying currents can also be produced by some 1P/6TM voltage-gated channels with a particular type of gating behavior – rapid, voltage-dependent inactivation [34, 35]. Such channels have limited outflow of K^+ ions at positive potentials because they rapidly enter an inactive state after opening in response to depolarization. When the membrane repolarizes, the channels recover from inactivation and reopen as they return to the closed state. During the reopened period large inward currents are seen as K^+ ions move down their electrochemical gradient into the cell. These channels are present in cells of widely disparate origin including the guard cells of plants [36] and mammalian cardiac myocytes [35].

Ligand-gated K^+ channels

Some K^+ channels are classified by the molecules that regulate their function. Three examples include calcium-activated K^+ channels that are opened by elevations in intracellular calcium or calcium and voltage in concert; these contain 1P/6TM subunits [37, 38]. ATP-sensitive K^+ channels are blocked by ATP, activated by ADP, and appear to act as sensors for the metabolic state of the cell; these channels are heteromultimers of 1P/2TM subunits and regulatory sulfonylurea receptors, members of the ABC transporter family [39] and carry ligand binding sites on both subunit types [40]. Acetylcholine-sensitive K^+ channels change their open probability in response to binding $G_{\beta\gamma}$ liberated by muscarinic receptor stimulation; these channels are heteromultimers of 1P/2TM channel subunits [41].

Accessory subunits

Single P domain subunits function in macromolecular complexes containing one or more accessory subunits. Required for integrated channel function, accessory subunits regulate channel expression levels, modify gating kinetics, mediate responses to ligands, and in some cases are required for activity [42]. Thus Kβ subunits speed inactivation of some voltage-gated delayed rectifier K^+ channels [43, 44], calcium-activated K^+ channel β subunits increase sensitivity to calcium [45], and ATP-sensitive channels operate when their P domain subunits aggregate with sulfonylurea receptors [46].

The P domain

Pores are the catalytically active sites of ion channels, and identification of P domains was a major step in understanding the structural basis for channel function [47–51]. The P domain residues in Shaker K^+ channels were found by systematic point mutation in an effort to

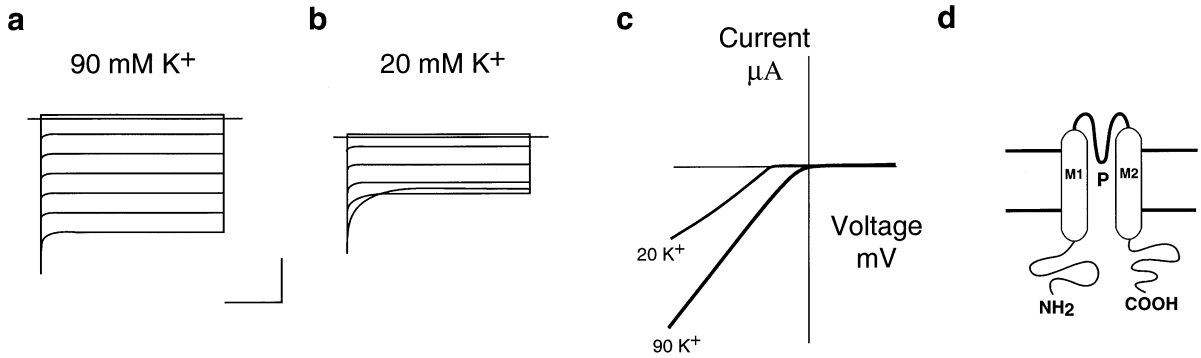

Fig. 2a–d Drawings to represent common attributes of inward rectifier K^+ channels. **a** Whole cell currents in high levels of external KCl (90 mM) in response to a family of voltage pulses from a holding voltage of 0 mV to command voltages of –160 to +50 mV in 30-mV steps. **b** In 20 mM external KCl. **c** Steady-state current/voltage relationships in 90 and 20 mM external KCl. **d** Probable 1P/2TM membrane topology. *Scale bars*, 1 μA and 5 ms

a

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Shaker     I P D A F W W A V V T M T T V G Y G D M T P

TOK   P1   Y G N A L Y F C T V S L L T V G L G D I L P
TOK   P2   Y F N C I Y F C F L C L L T I G Y G D Y A P

ORK   P1   F Y H A F F F A F T V C S T V G Y G N I S P
ORK   P2   S S I S L Y Y S Y V T T T T I G F G D Y V P

TPKC  P1   L G S S F F F A G T V I T T I G F G N I S P
TPKC  P2   A L D A I Y F V V I T L T T I G F G D Y V A

HOHO  P1   F T S A L F F A S T V L S T T G Y G H T V P
HOHO  P2   F L E S F Y F C F I S L S T I G L G D Y V P
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b

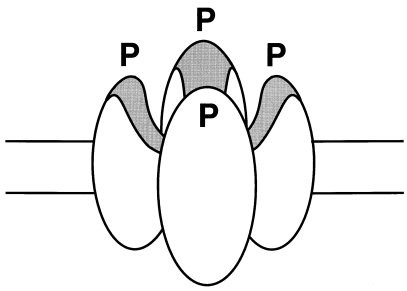


Fig. 3a, b P domain amino acid sequences and model for tetrameric pore formation. **a** Residues of the P domains in Shaker, Tok1, d-ORK1, h-TPKC1, and HOHO1 K^+ channel subunits (Genbank accession numbers M17211, U28005, U55321, AF004711, and U76996, respectively); *underlined*, the P domain *signature sequence* residues in Shaker [53]. **b** A drawing to suggest how four P domains may form the pore lining in voltage-gated and inward rectifier K^+ channels

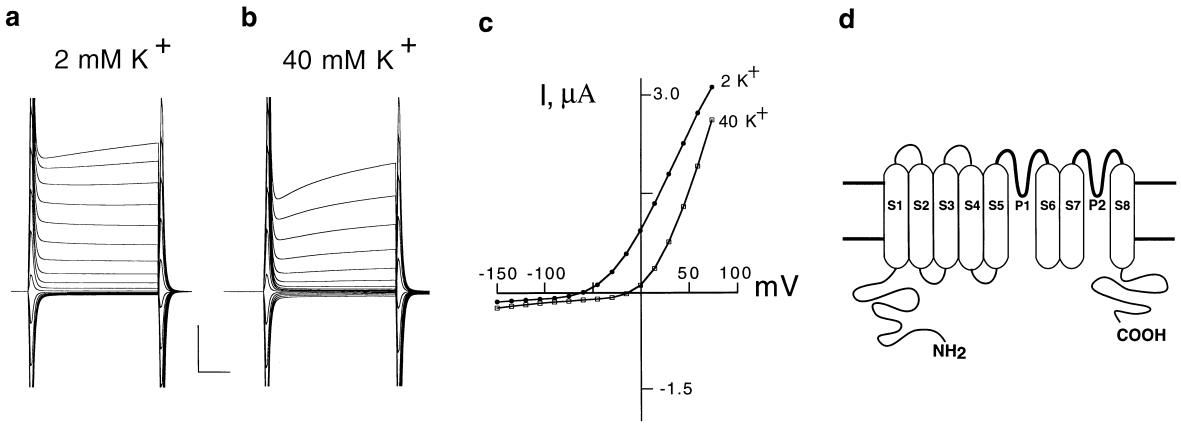
disrupt high-affinity pore blockade by a scorpion toxin [48]. That residues linking the fifth and sixth transmembrane segments enter the membrane to form the ion conduction pathway was shown by two approaches. First, two binding sites in this region were identified for the pore blocker tetraethylammonium ion, one extracellular and one intracellular [49, 50]. Second, a channel carrying transplanted linker residues exhibited pore characteristics of the donor including single-channel conductance and affinity for internal and external tetraethylammonium ion [51]. Later a "signature sequence" of eight highly conserved residues that are critical to K^+ ion selectivity was identified in the P domain, TxTTxGYG [52, 53]. Figure 3a shows the residues found in the P domain of Shaker as well as those in examples from each of the four known subfamilies of two P domain K^+ channels.

Site-directed mutagenesis has revealed that K^+ channel subunits with a single P domain associate as tetrameric aggregates [54, 55]. In these channels the ion conduction pathway is lined by four P domains and portions of the adjacent hydrophobic transmembrane stretches (Fig. 3b) [56–58]. Voltage-gated Na^+ and Ca^{2+} have a similar fourfold pseudosymmetric pore [1]. These larger proteins carry four homologous domains, each like a complete voltage-gated K^+ channel subunit with a P domain between every fifth and sixth membrane-spanning segment, and the four P domains fold together to form a central conduction pore. While the three-dimensional structure of intact K^+ channels has not yet been determined, the K^+ selectivity filter appears to form through association of pore "loops" like those seen in the bacterial porins [59].

Tok1, novel structure and function

Tok1, was the first K^+ channel identified to carry two P domains in one continuous polypeptide [11]. The gene was identified on *Saccharomyces cerevisiae* chromosome X by searching with the BLAST algorithm the data collected by the yeast genome sequencing project and available through the National Center for Biotechnology Information (NCBI). The Tok1 gene encodes a protein of 691 amino acids with two P domains and eigth probable transmembrane segments (Fig. 4c). When expressed in *Xenopus laevis* oocytes, it produced a current unlike any previously cloned channel but reminiscent of a current described in guard cells of the plant species, *Vicia* [60].

Fig. 4a–d Tok1 K^+ channels. **a** Currents measured by two electrode voltage clamp in an oocyte injected 2 days previously with 1 ng *TOK1* cRNA and bathed in 2 mM external KCl with voltage pulses of 100 ms from a holding voltage of –80 mV to command voltages of –150 to +45 mV in 15-mV steps. For method details see [11]. **b** Currents measured in 40 mM KCl. **c** steady-state current/voltage relationships for measurements shown in **a** and **b**. **d** proposed 2P/8TM membrane topology. *Scale bars*, 1 μA and 25 ms



Tok1 is an *outward rectifier* that preferentially passes outward K^+ currents in a fashion coupled to changes in the external K^+ concentration (Fig. 4c). This behavior is similar to that of inward rectifier channels but in the reverse direction (Fig. 2c). It is unlike the behavior of voltage-gated channels, whose gating depends on transmembrane potential in an invariant fashion (Fig. 1c). The macroscopic kinetics of Tok1 currents reveal two components, one rapid, the other time dependent (Fig. 4b) [11, 61, 62].

Much remains to be discovered about Tok1. The mechanism underlying outward rectification is as yet unknown. Tok1 is expressed in the plasma membrane of yeast cells [63] and regulated by protein kinase C and intracellular pH [61], but its functional role in vivo is undetermined. Based on the tetrameric structure of single P domain channels, it seems likely that dimers of Tok1 will be shown to form a single conduction pore, but this also requires experimental confirmation.

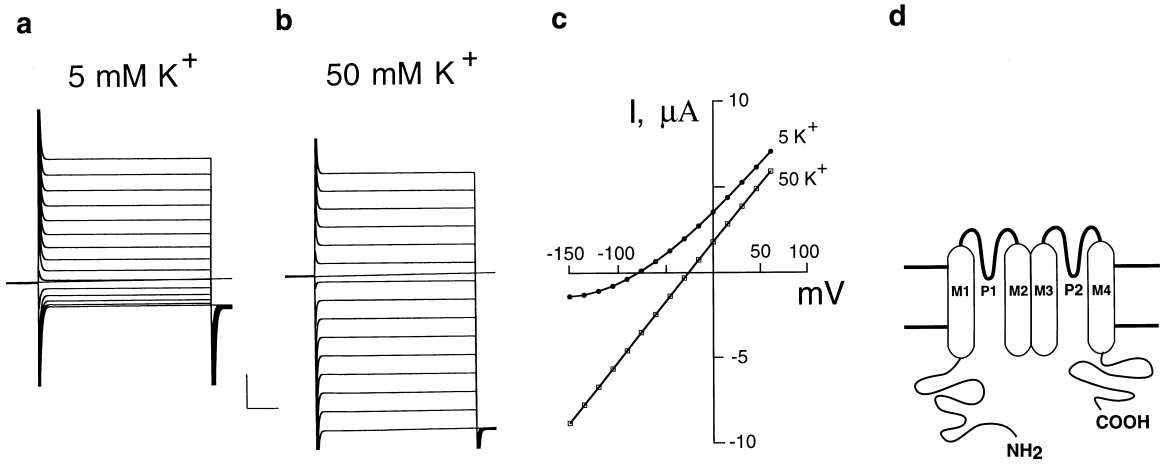
d-ORK1, a second lineage of two P domain channels with just 4 probable TM segments

d-ORK1 is a K^+ channel with two P domains expressed in the neuromuscular tissues of *Drosophila melanogaster* [10]. The gene was isolated from a cDNA expression library produced from fly larvae based on its ability to complement a strain of yeast defective in K^+ transport. Both the high- and low-affinity K^+ transport proteins (Trk1, Trk2) are disrupted in the yeast cells, limiting their ability to grow in low-K^+ medium. Expression of d-ORK1 in this strain allows the cells to survive in low-K^+ medium and confers the ability to accumulate K^+ ions. Based on its nucleotide sequence and glycosylation pattern, the channel protein appears to contain two P domains and only four transmembrane segments [10].

When expressed in *Xenopus* oocytes, d-ORK1 behaves as an open rectifier (or leak) K^+ channel, that is, currents behave as predicted for an open K^+-selective pore in an electric field. Currents are instantaneous with changes in voltage and rectify when transmembrane K^+ concentrations are unequal (Fig. 5a). These attributes are similar to those of the unidentified leak K^+ current underlying resting membrane potential in myelinated nerves of vertebrates [64].

d-ORK1, like Tok1, is still poorly understood. How do complete channels form? How are they regulated? Do d-ORK1 channels in fact mediate cellular resting potential? To address this question agents that act on the channel are being identified in rapid, large-scale screens exploiting channel-dependent growth of yeast cells (see [65]). Identified agents will later be used to evaluate the channels' role in vivo. Indeed, the ability to functionally express d-ORK1 in yeast cells allows application of a wide array of powerful genetic tools to study its structure, regulation, and function [66].

Fig. 5a–d d-ORK1 K^+ channels. **a** Currents measured by two electrode voltage clamp in an oocyte injected 2 days previously with 0.2 ng d-ORK1 cRNA and bathed in 5 mM external KCl with voltage pulses of 100 ms from a holding voltage of –80 mV to command voltages of –150 to +60 mV in steps of 15 mV. For method details see [10]. **b** Currents measured in 50 mM KCl. **c** Steady-state current/voltage relationship in **a** and **b**. **d** Proposed 2P/4TM membrane topology. *Scale bars*, 2 μA and 25 ms



h-TPKC1, an outward rectifier from human brain related to d-ORK1

A degenerate PCR approach (using primers based on the P domain sequences of d-ORK1 and several putative two P domain channels in the genome of *C. elegans*) were used to clone h-TPKC1, so named as an abbreviation for two P domain K^+ channel, from a human brain cDNA library [13]. That h-TPKC1 encodes a functional 2P/4TM K^+ channel was deduced from its expression in *Saccharomyces cerevisiae* cells defective for K^+ uptake which survived on low-K^+ medium as a result. Evaluation of the channel when expressed in *Xenopus* oocytes using two electrode voltage clamp revealed a largely instantaneous, noninactivating, K^+-selective outward current when measured at physiological levels of external K^+ (approx. 5 mM). As bath K^+ levels increased, the current reversal potential shifted toward E_K, in accordance with

the Nernst equation, and only a modest inward potassium current was detected (data not shown). These properties are held in common with those of Tok1, indicating that outwardly rectifying channel behavior does not require a 2P/8TM structure. A mouse homolog of h-TPKC1 was cloned while this work was in progress and is called mTREK1 [67]. The channels' are highly similar (85% identical) and exhibit common biophysical properties. Despite their similarity, their tissue and brain region specific pattern of expression differ substantially. Northern blotting has revealed that the strongest h-TPKC1 signal corresponds to a 3.8-kb brain transcript found at low steady-state levels in skeletal muscle, small intestine, and colon but undetectable in lung, kidney, and heart. In contrast, substantial mTREK1 mRNA is present in brain, lung, kidney, and heart.

HOHO1, another human homolog of d-ORK1

A search of the NCBI database of expressed sequence tags with the nucleotide sequence for d-ORK1 repeatedly identified a homologous sequence in humans. We isolated HOHO1 (for *h*uman *O*RK-*h*omologous *o*pen reading frame), from human brain cDNA (NCBI accession no. U76996). The protein has 337 amino acids and, as with d-ORK1, a probable 2P/4TM topology. HOHO1 is identical in its coding region to a human kidney protein, TWIK1 [12], and similar to a murine clone, mTWIK1 [68]; the TWIKs are reported to behave as inwardly rectifying K^+ channels when expressed in *Xenopus* oocytes. We have observed no currents by two-electrode voltage clamp when studying oocytes injected with 1–10 ng cRNA encoding HOHO1 in constructs that yield high expression of Tok1, d-ORK1 and h-TPKC1 [10, 11, 13]. This suggests that HOHO1/TWIK1 may need to associate with other channel subunits, is only a partial clone, or is subject to regulatory influences that vary between oocyte preparations.

Conclusions

Since the cloning of Shaker in 1988, over 100 K^+ channels with a single pore-forming P domain on each subunit have been identified [6]. This has revealed the molecular basis for many K^+ currents essential to normal physiology and allowed elucidation of the mechanism underlying a number of human diseases [24, 25, 30, 69, 70]. Now, a new superfamily of K^+ channels containing two P domains has emerged. Members of the new superfamily appear likely to be as numerous as their single P domain cousins. In the 2 years since the cloning of *TOK1* [11] three additional subtypes of two P domain channels have been identified which carry four rather than eight probable transmembrane segments. Database searching reveals this 2P/4TM subfamily to be common in the genomes of many organisms, indeed just the genome of the nematode *C. elegans* shows over 20 predicted open reading frames with this pattern [71, 72].

These findings herald four changes in the field of K^+-selective ion channels. First, increasingly rich databases for genomic and expressed nucleotide sequences are changing the way in which genes for ion channels are identified, cloned, and studied. Second, it is now clear that K^+-selective ion channels can be formed by association of protein subunits with either one or two P domains; how their structures are similar and how unique remains to be revealed. Third, the functional repertoire of cloned K^+ channels is growing; although two P domain channels share attributes common to known K^+ channels, such as high selectivity for potassium, they also exhibit novel phenotypes, such as outward rectifier and open (or leak) K^+ currents. Fourth, powerful new methods for exploring the molecular basis for function of K^+ channels from animals are now feasible through expression of K^+ channels in yeast cells [73]. It is premature to conjecture the roles two P domain channels will play in human health and disease. However, by determining the place of two P domain channels in normal physiology we may discover new targets for the development of therapeutic agents [73] and uncover clues to the molecular basis for disorders that result from ion channel dysfunction [15].

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